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Evidence for the existence of multiple heparan sulfate proteoglycans in the human glomerular basement membrane and mesangial matrix

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Heparan sulfate proteoglycans (HSPGs) are essential components of the glomerular basement membrane (GBM) carrying a strong anionic charge. A well-characterized extracellular HSPG is perlecan, ubiquitously expressed in basement membranes. A cDNA construct encoding domains I and II of human perlecan was expressed as a fusion protein with glutathione *S*-transferase. This fusion protein was used to generate monoclonal antibody 95J10. We compared the staining pattern of 95J10 with that of M215, a previously prepared mAb that recognizes HSPG isolated from human GBM. In kidney cortex, the anti-perlecan mAb 95J10 showed a strong staining of the mesangium, Bowman's capsule, the tubular basement membrane, and stained the GBM only slightly. In contrast, M215 predominantly stained the GBM in a linear fashion. Immunoelectron microscopy supported these results, showing concentrations of perlecan in some regions of the GBM, whereas the unidentified M215 antigen was homogeneously distributed throughout the GBM. In other human tissues, both antibodies also produced a different staining pattern. Furthermore, a polyclonal antiserum recognizing HSPG isolated from the GBM did not recognize perlecan from EHS tumors. These results provide evidence for the presence of another HSPG in the GBM that is immunologically distinct from perlecan. The absence of perlecan splice variants in the kidney suggests that this component is encoded by a different gene than perlecan. Given its marked expression in the GBM, this component could be a determining factor in the maintenance of selective glomerular permeability.

Keywords: perlecan; heparan sulfate proteoglycan; prokaryotic expression; glomerular basement membrane.

The glomerular basement membrane (GBM) is a specialized extracellular matrix located at the interface of the glomerular visceral epithelium and the vascular endothelium. The GBM provides a rigid structure that supports the tissue to withstand high local blood pressure, its mechanical strength being enhanced by the highly organized interplay of its biopolymer components [e.g. collagen IV, laminin, nidogen, and heparan sulfate proteoglycan (HSPGs)] [1]. Another crucial function of the GBM is to regulate the passage of proteins depending on their molecular size and charge [2]. The impermeability of the GBM for anionic macromolecules is assigned to the presence of HSPGs anchored within it. The electrostatic charge of these HSPGs can be visualized by electron microscopy using cationic ferritin [2] or heparan sulfate (HS)-specific mAbs [3]. The significance of this mechanism was demonstrated by perfusing rat

kidney with heparitinase, which resulted in leakage of anionic tracer proteins [4]. A similar effect was observed when the kidney was perfused with anti-HS mAbs [5]. Alterations in the HS content of the GBM causing nephrotic syndrome are involved in, for example, minimal change nephropathy, glomerulonephritis, diabetes mellitus, and Denys-Drash syndrome [6–8].

For biochemical characterization, HSPGs were extracted from isolated glomeruli [9], from epithelial cell lines [10], or from the mouse Engelbreth-Holm-Swarm sarcoma [11–13]. Extraction of HSPG from basement membranes is complicated due to its strong interactions with other components. In addition, their analysis is confused by their smeared appearance on protein gels and their sensitivity to proteolysis. The first basement membrane proteoglycan characterized was termed perlecan, with reference to its beads-on-a-string-like appearance in rotary shadowing electron microscopy [14]. It consists of a 467-kDa core protein carrying five functional domains and three HS chains attached in close proximity to its N-terminus [15–17]. Human perlecan is expressed by a single gene, named *HSPG2*, located on the short arm of chromosome 1 [18–20]. Several observations have suggested the presence of additional basement membrane HSPGs: low-density forms (representing perlecan) and high-density forms characterized by relatively small core proteins (18–143 kDa) [21, 22]. However, it was demonstrated that these high-density proteoglycans are immunologically related to

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Abbreviations. GBM, glomerular basement membrane; GST, glutathione *S*-transferase; HS, heparan sulfate; HSPG, heparan sulfate proteoglycan.

Enzymes. Glutathione transferase (EC 2.5.1.18); DNA ligase (ATP) (EC 6.5.1.1); thrombin (EC 3.4.21.5); type II site-specific deoxyribonuclease (EC 3.1.21.4).

perlecan, suggesting that they are generated by proteolytic processing [23].

Previous immunofluorescence studies performed with various polyclonal and monoclonal antibodies yielded inconsistent results, with particular differences in the staining intensity of the GBM [3, 9, 11, 24, 25]. For the generation of mAbs, prokaryotic expression offers a large advantage over proteoglycan extraction from basement membranes, because the risk of co-immunization with contaminating HSPG species is eliminated. Furthermore, recombinant protein expression allows the development of domain-specific antibodies. We have synthesized domains I and II of human perlecan as a bacterial fusion protein and generated monoclonal antibody 95J10. In this study, we compare the expression pattern in human tissues for 95J10 and M215, a mAb that was previously raised against isolated human GBM-HSPG [3]. The results provide evidence for the presence of an unidentified HSPG in the GBM, which appears to be immunologically distinct from perlecan.

EXPERIMENTAL PROCEDURES

Materials. The following were obtained from the indicated sources: pGEX4T-3 expression vector, glutathione-conjugated Sepharose 4B (Pharmacia Biotech); reduced glutathione (Boehringer Mannheim), pCR-Script cloning vector, *SrfI* (Stratagene Cloning Systems); isopropylthio- β -D-galactoside, T4 DNA ligase, *NcoI*, *BamHI* (Life Technologies); Vent polymerase, *NotI*, *XcmI* (New England Biolabs); thermal cycler apparatus (Perkin Elmer); automated sequencer (Applied Biosystems); thrombin, phenylmethylsulfonyl fluoride, ribonuclease A (Sigma), 2-iodoacetamide (Merck); *N*-ethylenmaleimide, EDTA (Fluka Chemie); benzamidinium hydrochloride (Janssen Chimica); 6-aminohexanoic acid (Aldrich); Tween-20 (ICN Biomedicals); Immobilon-P, ultrafiltration units (Millipore); goat-anti-mouse peroxidase conjugate (Dakopatts); sheep-anti-mouse fluorescein isothiocyanate conjugate (Cappel, Oss, The Netherlands); [γ - 32 P]ATP, ECL detection kit (Amersham).

Construction of the expression vector pGEX4T3-P12a. A prokaryotic expression vector encoding amino acids 24–404 of human perlecan was prepared as follows. Two PCR products were generated from cDNA clone Hpe2 [16] using Vent polymerase for proofreading activity. The primer sets were: 5'-TCA-TCTCCAGCGGCTCTGT-3' (forward P1), 5'-TCATGGGAAC-TGGGGCACTGTGC-3' (reverse P1), 5'-TCATCTCCAGCGG-CTCTGT-3' (forward P2) and 5'-TTACATGCAGCCAACTC-GTC-3' (reverse P2, this primer introduces an in-frame stop codon immediately behind amino acid 404). The two PCR products were inserted into the *SrfI* cloning site of pCR-Script [26, 27] and screened to detect the unique *NotI* site at the 3'-end of the cDNA fragments. The P2 fragment was then ligated to the P1 fragment by use of the *XcmI* restriction site, introduced into pGEX4T-3 [28] using the *NcoI* and *NotI* cloning sites. The fidelity of amplification and ligation procedures was checked by sequence analysis. The resulting expression vector was named pGEX4T3-P12a.

Expression and purification of recombinant perlecan-(24–404)-peptide. Expression of the recombinant perlecan-(24–404)-peptide was performed as described in [28], except that the cell pellet was resuspended in 125 ml ice-cold NaCl/P_i (140 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄, pH 7.5), 1% Triton X-100, 10 mM *N*-ethylmaleimide, 5 mM EDTA, 10 mM 6-aminohexanoic acid, 5 mM 2-iodoacetamide, 1 mM phenylmethylsulfonyl fluoride and 1 mM benzamidinium/HCl. Cell lysis and affinity purification of the recombinant fusion protein were performed in the presence of 1% Triton X-

100, which was essential for optimal yields. In contrast, the yield could not be improved by other modifications of the procedure such as the use of sarkosyl for solubilization [29], growth at 30°C [30] or treatment with Mg²⁺ and ATP prior to affinity chromatography [31].

Affinity-purified fusion protein was incubated with 1.7 NIH U/ml human thrombin (specific activity approximately 4000 NIH U/mg) and incubated for 60 min at 22°C. To exhaustively remove the thrombin-digested mixture from the glutathione S-transferase (GST) fragment, it was dialysed against NaCl/P_i to remove free glutathione and passed through a glutathione-conjugated Sepharose 4B column (1 ml) at room temperature. The flow-through was concentrated by ultrafiltration against a 10-kDa cutoff membrane to a final concentration of 0.63 mg/ml (determined by the Bradford method with BSA as reference) [32].

Antibodies. The anti-perlecan mAb 95J10 was prepared as follows. Mice were immunized and boosted with 20 μ g purified perlecan-(24–404)-peptide per injection (this preparation was completely devoid of GST). Hybridoma cell lines were screened by ELISA with an independently isolated batch of recombinant fusion protein. Twelve positive clones were examined in indirect immunofluorescence studies for their capacity to stain human renal basement membranes. A mAb designated 95J10 was selected that showed strong glomerular staining. The mAb also reacted in western blot analysis with the fusion protein and with perlecan-(1–404)-peptide expressed by baculovirus-infected insect cells [33], which indicated that it also recognizes the antigen in a denatured conformation. No cross-reactivity of mAb 95J10 with *Escherichia coli* proteins or GST was observed by ELISA and western blotting. Experimental animal care, cell fusion, and generation of the monoclonal hybridoma cell line 95J10 were performed by Diabor Ltd, Oulu, Finland.

Other antibodies used were R254 (a rat mAb against mouse perlecan; a generous gift of Dr J. van den Born), M215 (a mouse mAb against the core protein of HSPG isolated from human GBM [3]), and K42 (a rabbit antiserum recognizing HSPG from the human and murine GBM [9]).

Immunological methods. SDS/PAGE and immunoblot analyses were carried out following standard procedures [30]. All samples were denatured by boiling in the presence of 2-mercaptoethanol prior to loading onto a homogeneous 5% polyacrylamide gel. Immunofluorescence and tissue distribution studies were performed with adult human tissue specimens obtained during autopsy, snap-frozen in liquid nitrogen, and stored at –80°C until use. Cryostat sections of 2 μ m were attached to glass slides and treated with acetone for 10 min at 4°C for fixation. mAb M215 or 95J10 was applied as undiluted hybridoma supernatant. After 1 h incubation at 22°C, specimens were submerged five times in fresh NaCl/P_i for washing. Bound primary antibody was detected by incubating with fluorescein-isothiocyanate-conjugated sheep-anti-mouse immunoglobulins (30 min at 22°C in the dark). After thorough washing with fresh NaCl/P_i, the preparations were examined with a Leitz immunofluorescence microscope. For immunoelectron microscopy, kidney cortex slices were fixed with periodate-lysine and paraformaldehyde, immersed in 2.3 M sucrose for cryoprotection and snap-frozen in liquid nitrogen. Staining of peroxidase-conjugated antibody complexes was performed following standard protocols [34] and the sections were postfixed in Epon 812. Thin sections were examined with a Jeol 1200 EX electron microscope.

Northern hybridization. A multiple human tissue northern blot (Clontech Laboratories Inc.) was treated according to the supplier's protocol with an antisense probe homologous to exon 3 of perlecan (5'-AAGTCCCCGCTGCCAGGTCCCCACTGCCAGGTCGT-3') [20]. This exon is a prerequisite for the

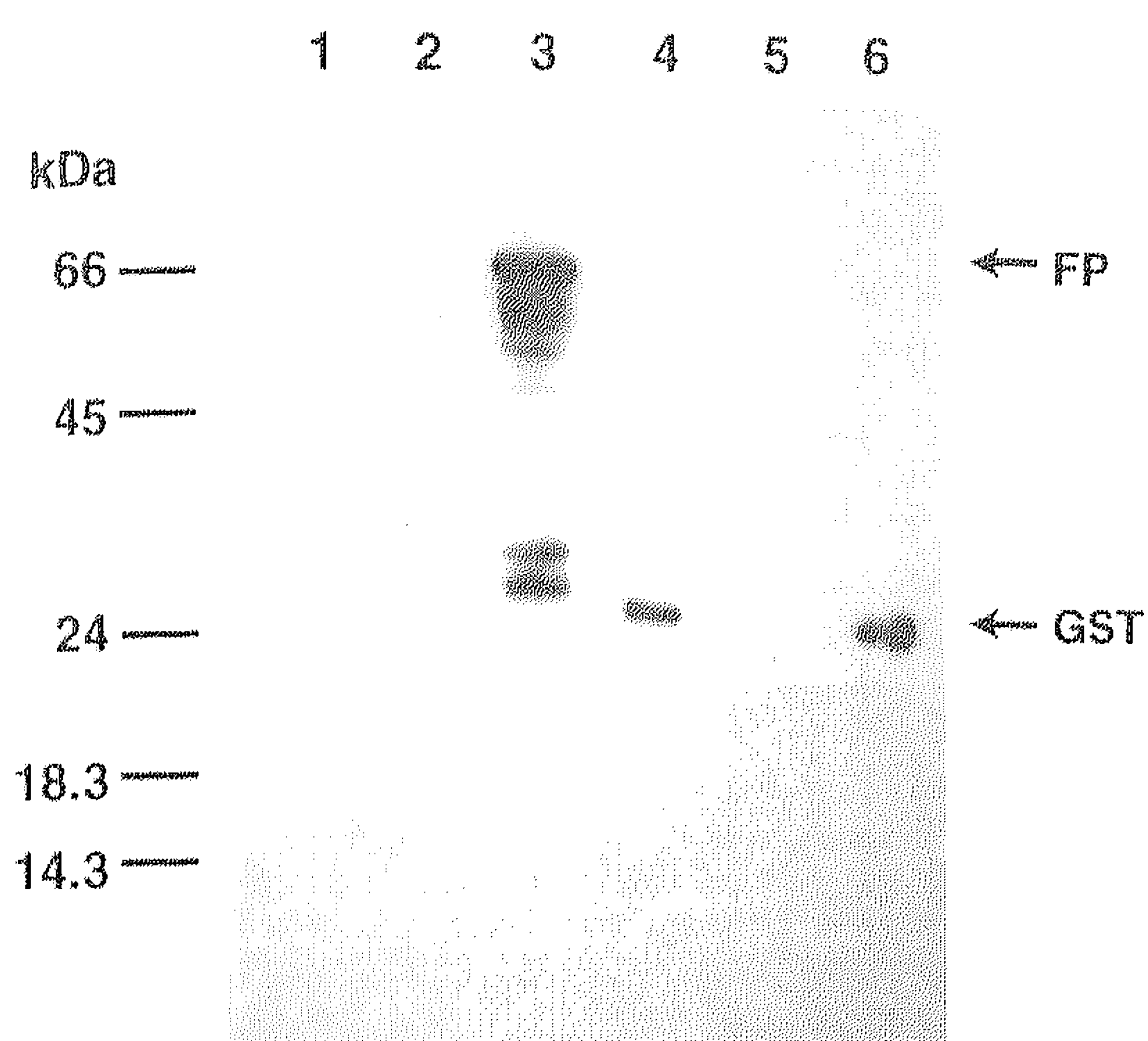


Fig. 1. Western blot detection of recombinant fusion protein with a GST-specific monoclonal antibody. Lane 1, crude cell extract of bacteria carrying the plasmid pGEX4t3; lane 2, crude cell extract of bacteria carrying pGEX4t3-P12a; lane 3, affinity purified preparation of the fusion protein; lane 4, thrombin-digested fusion protein; lane 5, perlecan-(24–404)-peptide fragments obtained from thrombin-digested fusion protein; lane 6, GST fragment obtained from thrombin-digested fusion protein. The positions of marker proteins are given on the left (kDa). Arrows on the right indicate the fusion protein in lanes 2 and 3 (FP) and the GST fragment in lanes 4 and 6.

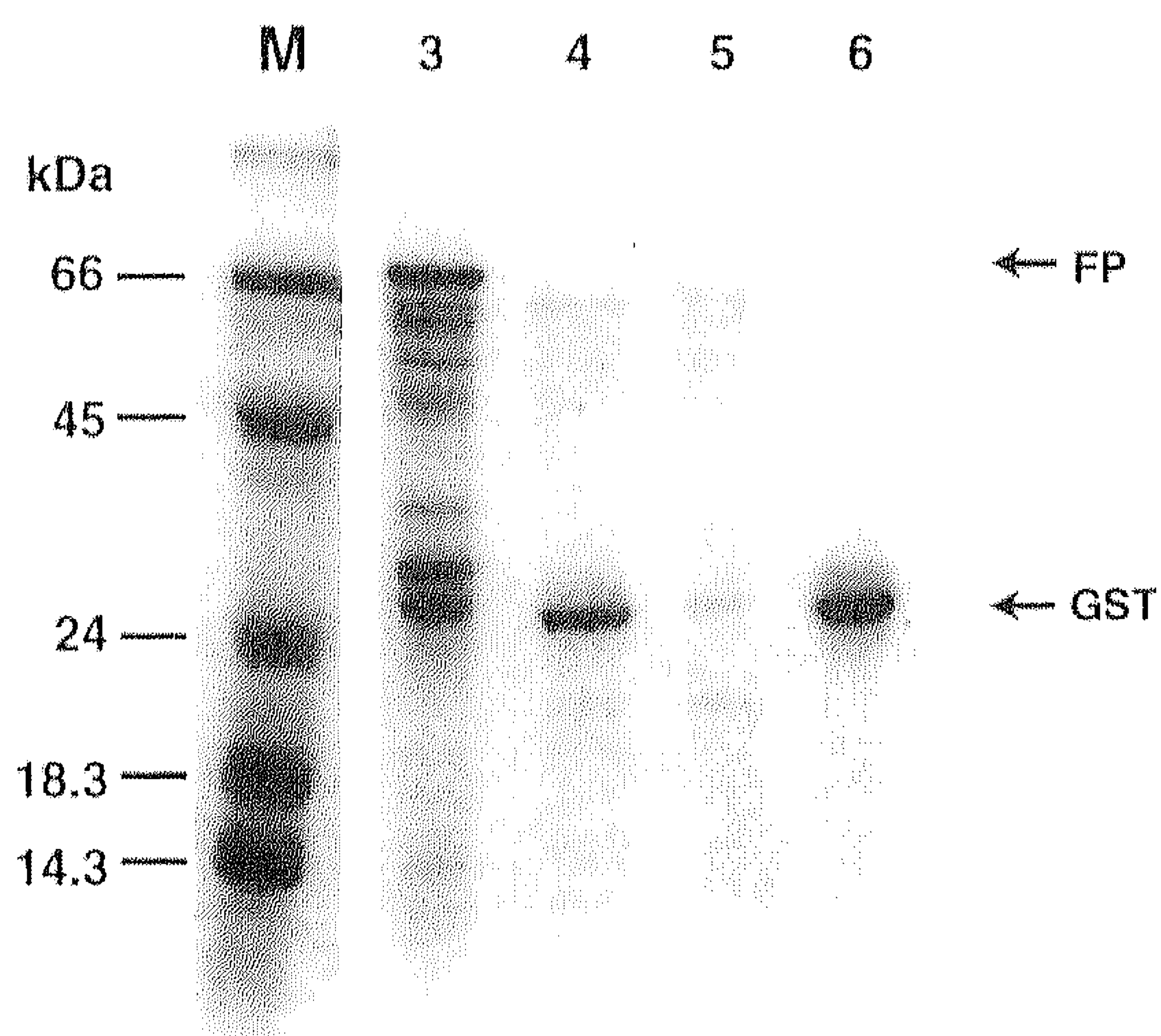


Fig. 2. SDS/PAGE analysis of recombinant protein produced by recombinant *E. coli*. Recombinant protein was extracted from *E. coli*, purified by glutathione affinity chromatography and digested with thrombin. Proteins were separated by SDS/PAGE (12.5% polyacrylamide) and stained with Coomassie brilliant blue. M, marker lane; molecular mass indications are given on the left (kDa). The contents of lanes 3–6 are as described in Fig. 1.

presence of the HS chains. After hybridization, the filter was washed for 3×15 min under stringent conditions (15 mM NaCl, 1.5 mM sodium citrate, 0.1% SDS, pH 7.0 at 50°C) and exposed for autoradiography for 91 h.

RESULTS

Expression of perlecan domains I and II as a fusion protein with GST. A cDNA construct was prepared encoding domains I and II of human perlecan, fused to the carboxyl end of GST from *Schistosoma japonicum* [28]. For localization of the recombinant protein, we tested the cell lysate, the culture supernatant,

and the inclusion bodies isolated from the bacteria. The recombinant protein was found exclusively in the cell lysate. Crude cell extracts were analyzed by SDS/PAGE and immunoblotting with an anti-GST mAb (Fig. 1), confirming the presence of a recombinant fusion protein. Although the fusion protein was expressed at a level of 1.3 ± 0.3 mg/l⁻¹ crude culture (mean \pm SD, $n = 4$), only 120 μ g/l could be recovered by affinity chromatography. The affinity-purified fraction did not appear as a single band on SDS/PAGE, but contained multiple proteins as shown in lane 3 (Fig. 1). The largest of these proteins appeared as a strong band, which corresponded to the predicted molecular mass of 68 kDa. The observed additional bands and the low final yield suggest sensitivity of the fusion protein to proteolysis.

The GST fragment was removed from the hybrid protein utilizing an engineered recognition site for thrombin cleavage, and an additional round of affinity chromatography. Coomassie brilliant blue staining of the recombinant perlecan-(24–404)-peptide preparation identified two bands of 26 kDa and 19 kDa in size (Fig. 2, lane 5). The staining signal is weak compared to the GST fraction (Fig. 2, lane 6), which indicated a relatively low protein concentration. These results likely reflect proteolytic degradation of the fusion protein, also observed in lane 3 (Figs 1 and 2). The final preparation, completely devoid of GST as shown in Fig. 1 (lane 5), was used to generate the monoclonal antibody mAb 95J10.

HSPG distribution in the human kidney. In indirect immunofluorescence studies on human kidney cortex, mAb 95J10 displayed a bright staining of the mesangium, Bowman's capsule, the tubular basement membrane, and peritubular capillaries (Fig. 3B). The overall staining of the GBM was weak and visible only along fragments of the glomerular capillaries. In the arteriole, an intense staining was observed in intima, media, and adventitia (Fig. 3). The staining was especially pronounced at the luminal border. The staining was compared with that of another mAb designated M215. This mAb was obtained by immunization with HSPG purified from isolated human GBM, and is directed against an epitope of the core protein [3]. mAb M215 showed a strong linear staining of the GBM in immunofluorescence studies (Fig. 3D), along with a less intense but also linear staining of all other renal basement membranes (Bowman's capsule, the tubular basement membrane, and the basement membranes of the peritubular capillaries). mAb M215 did not stain the mesangial matrix. In contrast to 95J10, vascular staining of M215 was restricted to the endothelial border (Fig. 3C).

The ultrastructural distribution of HSPGs in the GBM was analyzed by immunoelectron microscopy with both mAbs (Fig. 4). The anti-perlecan mAb 95J10 stained the GBM along some fragments of the capillary wall, whereas other regions showed no staining at all (Fig. 4A). In all cases, the staining was clustered at the endothelial side of the basal lamina (Fig. 4C). Besides the local accumulation of perlecan in the GBM, an elevated expression was seen in the mesangial matrix (Fig. 4A). M215, however, produced a homogenous staining along the entire length of the GBM (Fig. 4B and D). The clustered appearance of perlecan, and the linear distribution of non-perlecan GBM-HSPG, agree with the results from immunofluorescence studies.

HSPG distribution in human tissues. Adjacent sections of human tissues were stained for perlecan (mAb 95J10) and non-perlecan GBM-HSPG (mAb M215). We especially focused on tissues that clearly displayed different staining patterns in indirect immunofluorescence studies using both mAbs. Fig. 5 shows typical results in skin, pancreas, artery, tongue, and cerebrum. Both antibodies stained the epidermal basement membrane in the skin (Fig. 5A and F). In addition, mAb 95J10 stained the capillaries more intensely than M215. In the pancreas, mAb

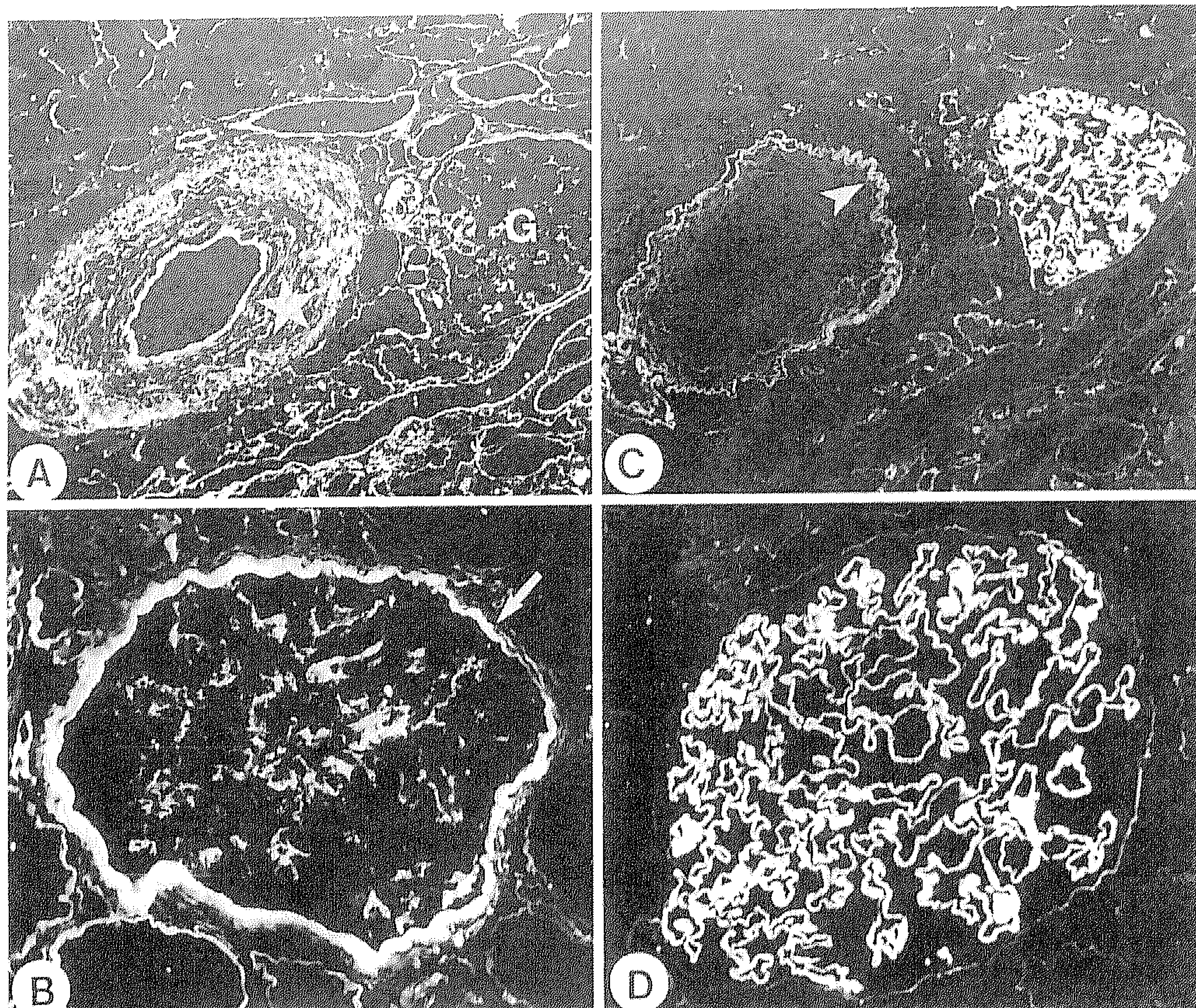


Fig. 3. Immunofluorescence detection of heparan sulfate proteoglycans in the human renal cortex. Cryosections were stained for perlecan (A, B) and non-perlecan GBM-HSPG (C, D) using the mAbs 95J10 and M215, respectively. The asterisk in A indicates the tunica media of the arteriole; the arrow in B indicates the Bowman's capsule; the arrowhead in C indicates autofluorescence typically originating from the elastica interna of the arteriole. Magnifications are 150 \times (A, C) and 400 \times (B, D).

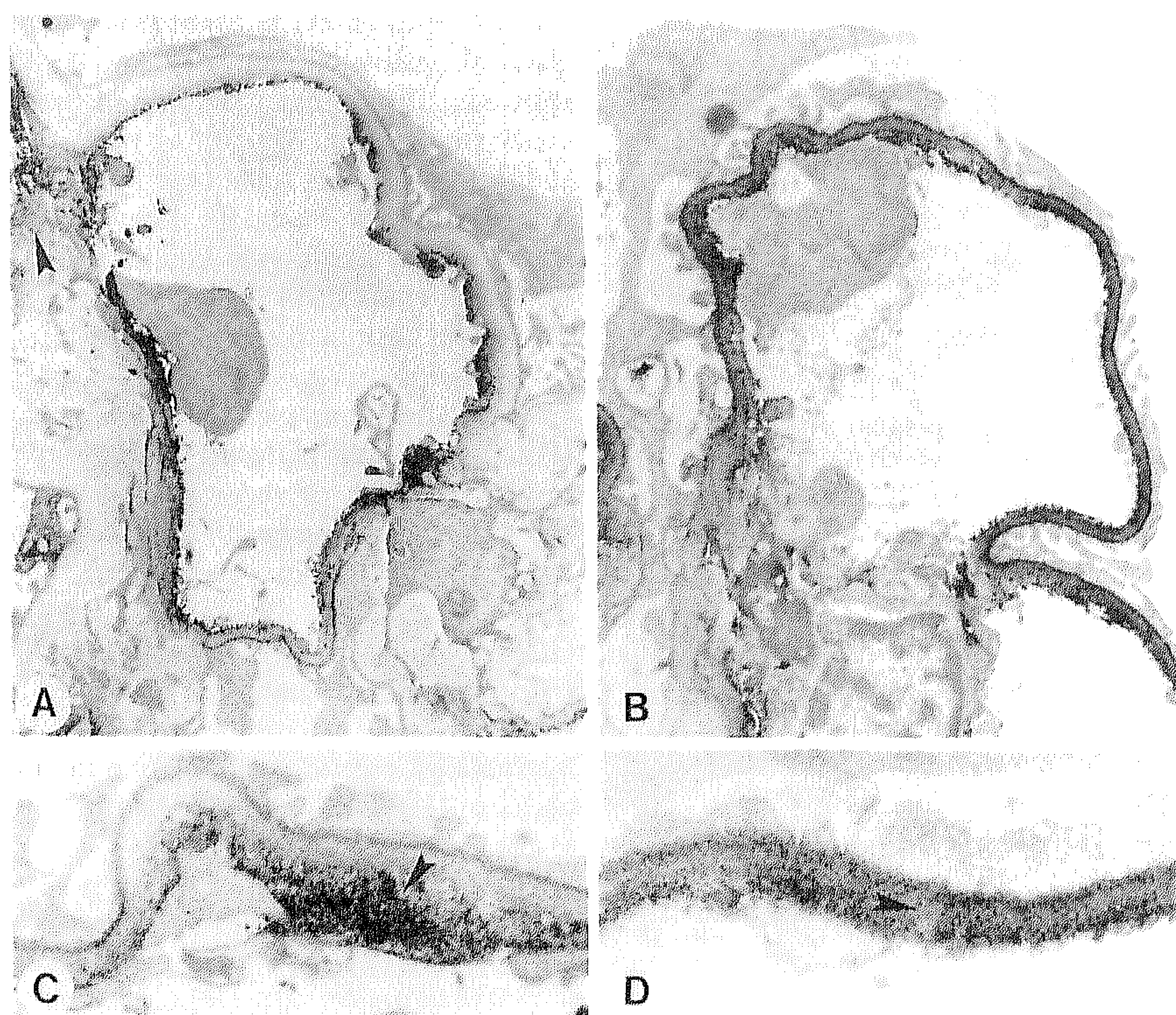


Fig. 4. The ultrastructural localization of HSPGs in the glomerular basement membrane. Human kidney cortex was stained for perlecan (A, C) and non-perlecan GBM-HSPG (B, D), using mAbs 95J10 and M215 and was examined by immunoelectron microscopy. The arrowhead in A shows elevated levels of perlecan in the mesangial matrix. The arrowhead in C shows a typical condensation of perlecan on the endothelial side of the GBM. The arrowhead in D shows the homogenous linear distribution of non-perlecan GBM-HSPG. Magnifications were 5000 \times (A, B) and 15 000 \times (C, D).

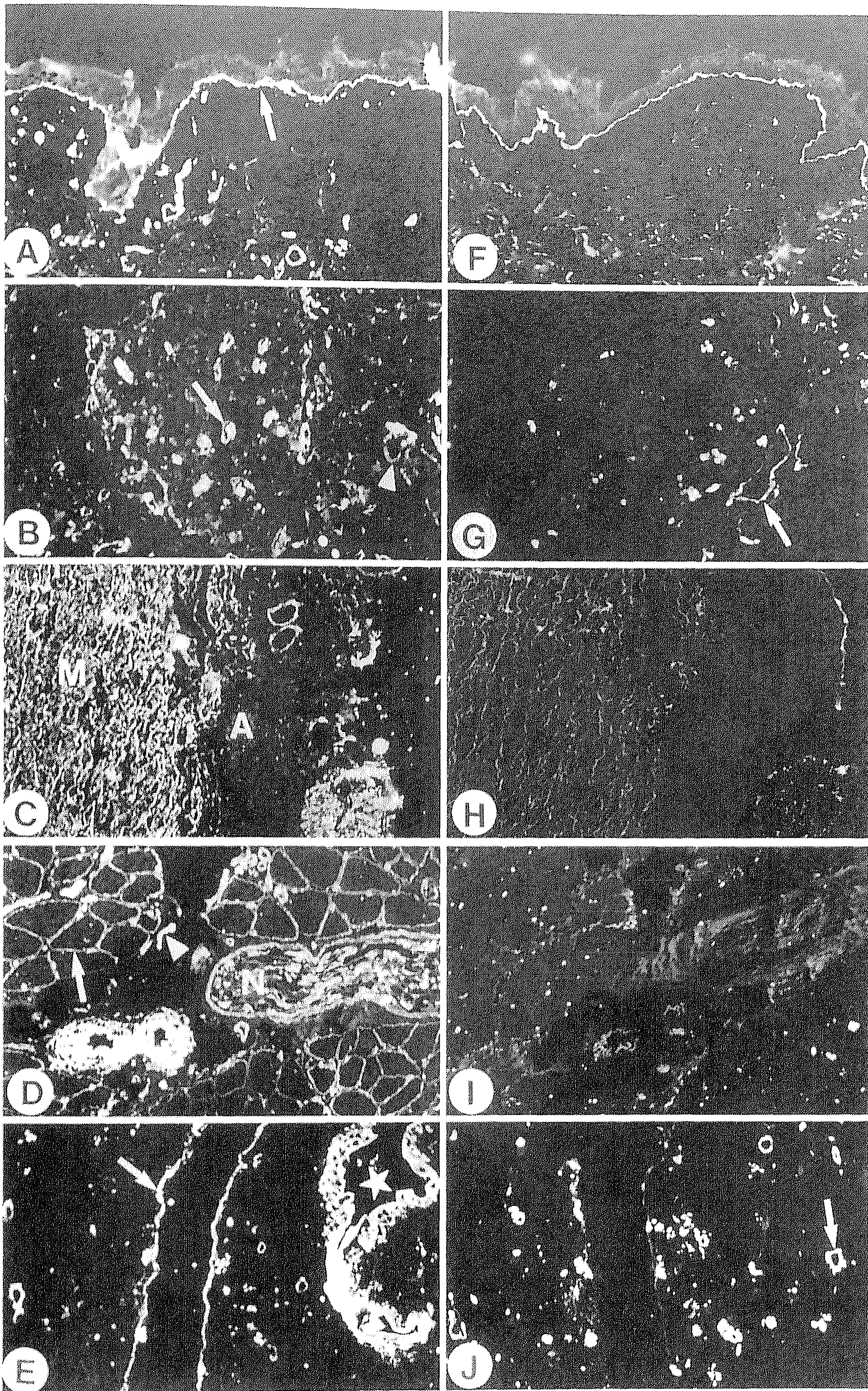


Fig. 5. Indirect immunofluorescence staining of HSPGs in various human tissues. Cryosections were stained with mAbs 95J10 (A–E) or M215 (F–J). A and F, skin (arrow indicates epidermal basement membrane); B and G, pancreas (in B arrow indicates sinusoids and arrowhead indicates capillary. In G, arrow indicates capillary). C and H, artery (M, media; A, adventitia). D and I, tongue (arrow indicates skeletal muscle fibers; arrowhead indicates capillary; N, nerve fibers). E and J, cerebrum (in E, arrow indicates vein; asterisk indicates artery. In J, arrow indicates capillary). Magnifications were 100 \times (C, E, H, J), 150 \times (A, D, F, I), and 400 \times (B, G).

95J10 (Fig. 5B) displayed a strong staining of the sinusoids, the islets of Langerhans, and the capillaries whereas mAb M215 (Fig. 5G) only stained the capillaries. In arteries, 95J10 (Fig. 5C) stained the media and adventitia with vasa vasorum,

reflecting the basement membrane distribution surrounding the smooth muscle cells. In contrast, the staining by mAb M215 was confined to the vasa vasorum (Fig. 5H). In cryosections from human tongue, mAb 95J10 (Fig. 5D) strongly recognized the

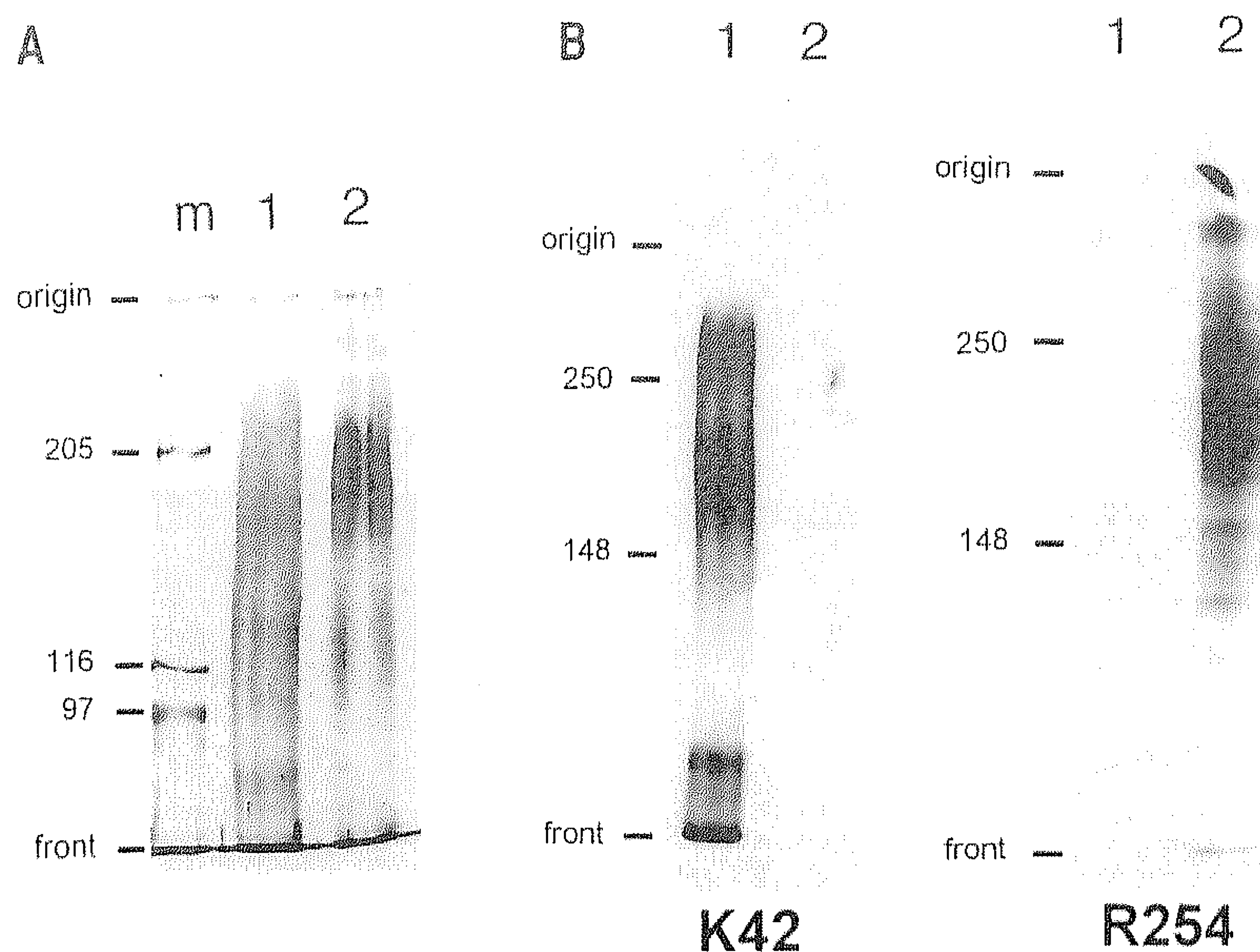


Fig. 6. Comparative immunoblot analysis of HSPGs from glomerular basement membrane and EHS tumour. Equal quantities of HSPG isolated from the human GBM (lanes 1) and from the mouse EHS tumor (lanes 2) were separated by SDS/PAGE (5% polyacrylamide). Both HSPGs were analyzed by silver staining (A) and immunoblotting (B). For immunoblotting, we used K42 (a rabbit pAb that recognizes GBM-HSPG from mouse and man; left part of B) and R254 (a rat mAb recognizing mouse perlecan; right part of B).

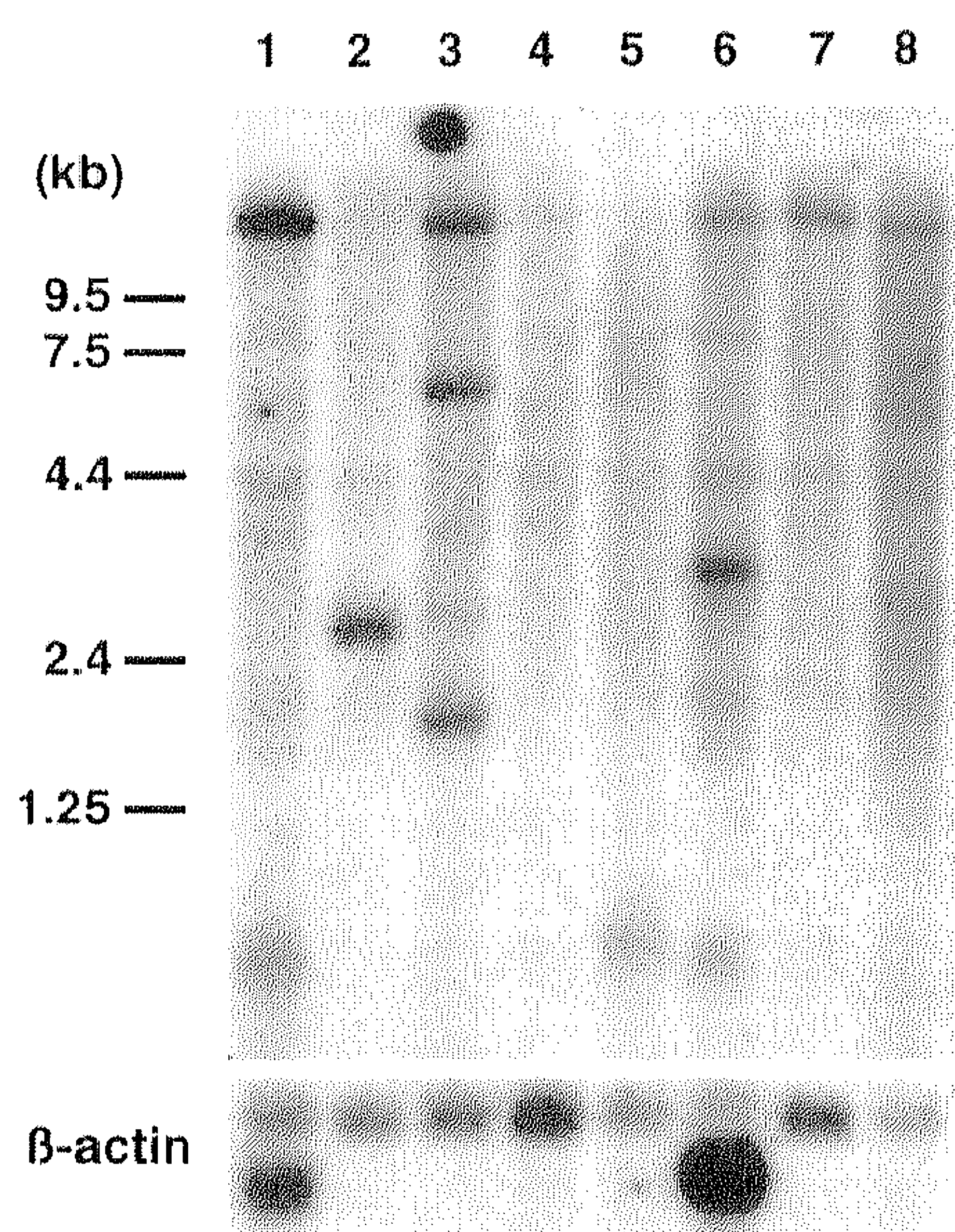


Fig. 7. Northern blot analysis of mRNA of various human tissues. Poly(A)-rich mRNA isolated from heart (1), brain (2), placenta (3), lung (4), liver (5), skeletal muscle (6), kidney (7), and pancreas (8) was hybridized with a perlecan-specific probe and washed stringently. The bottom panel shows a control hybridization with a β -actin-specific probe to indicate differences in total mRNA content.

endomysium that surrounds the skeletal muscle fibers, the capillaries, an artery, nerve fibers with a myelin sheath, and perineurium (bundles are surrounded by connective tissue). In contrast, mAb M215 stained only the capillaries (Fig. 5I). In cerebrum, 95J10 strongly stains veins in the cerebral cortex, the arteries, and the capillaries. In the same tissue, mAb M215 only stains the capillaries (Fig. 5J). In all cases, the specificity of the staining was checked by parallel incubations without the primary antibody.

The results obtained by immunohistochemistry suggest the occurrence of two different GBM-associated HSPG species. Could the observed differences be based on different epitope

accessibilities *in vivo*? To exclude this possibility, preparations of HSPG isolated from the human GBM and from the mouse EHS tumor were analyzed by western blotting (Fig. 6). The EHS tumor is known to produce large amounts of perlecan. Using a rabbit polyclonal antiserum (K42) that recognizes both murine and human GBM-HSPG [9], no staining of EHS-HSPG was detected. Staining with mAb M215 gave identical results (data not shown). As a control for the transfer of EHS-HSPG to the blot, an identical treatment with a rat mAb against mouse perlecan resulted in a marked staining of EHS-HSPG. mAb 95J10 could not be used for this purpose, since it is specific for human perlecan. The specificity of antiserum K42 for GBM-HSPG, without any cross-reaction with perlecan, confirms that the two HSPG species are immunologically unrelated.

Perlecan splice variants occur in brain, placenta and skeletal muscle but not in kidney. Elucidation of the perlecan gene structure showed the theoretical possibility of alternative splicing [16, 17, 20]. Interestingly, the occurrence of splice variants would result in the expression of perlecan isoforms. We therefore investigated the occurrence of perlecan splice variants by northern blotting (Fig. 7). The full size messenger RNA (approximately 14 kb) was detected in heart, placenta, skeletal muscle, kidney and pancreas, but was hardly or not at all present in brain, lung, and liver. Interestingly, a smaller size mRNA species was detected in brain (2.5–2.6 kb), placenta (1.9 kb and 6.4–6.7 kb) and skeletal muscle (3.1–3.3 kb). The significance of these molecules is not clear. In the kidney, however, we could not distinguish any splice variants.

DISCUSSION

As the presence of strongly anionic HSPGs is important to maintain the selective permeability of the GBM, the identification of perlecan as a basement-membrane-associated HSPG has provoked many investigators to study perlecan expression levels during nephropathy. We used mAb 95J10, directed against amino acids 22–404 of human perlecan, to analyze the distribution of perlecan in the glomerulus in more detail. The staining pattern of 95J10 in indirect immunofluorescence is in agreement

with other studies performed with mAbs raised against recombinant fragments from domain III of perlecan [24, 25], and with a polyclonal antiserum against perlecan from the EHS-tumor [11]. However, the exact localization of perlecan in the GBM can hardly be deduced from immunofluorescence microscopy studies. Our results from immunoelectron microscopy indicate that perlecan is present in the GBM, but only on the endothelial side. Moreover, perlecan is distributed in a non-homogenous pattern and many segments of the GBM were not stained by 95J10. Therefore, charge-selective properties of the GBM cannot be ascribed to the anionic charge of perlecan alone.

The expression pattern of perlecan is in marked contrast to that observed with M215, a previously described mAb against HSPG isolated from the GBM [3]. This HSPG is less widely distributed than perlecan, as shown by indirect immunofluorescence on multiple human tissues. In the kidney, this antibody predominantly stained the GBM in a linear manner. Similar results were obtained with other monoclonal and polyclonal antibodies raised against GBM-HSPG, and with a mAb that recognizes an epitope on the HS chain [5, 8, 9]. The homogenous distribution of this HSPG within the GBM was confirmed by immunoelectron microscopy.

Our results indicate that two immunologically unrelated HSPG species are present in the GBM. This conclusion is based on the following considerations. Firstly, the anti-HSPG antibodies 95J10 and M215 show a clearly different distribution in human tissues. Comparing the staining patterns of 95J10 and M215, it is of importance that both antibodies are directed against the core proteins. The exact composition of the glycosaminoglycan residues is not solely dependent on the nature of the core protein, but is also influenced by the cellular environment in which they are synthesized and modified. Secondly, we demonstrated that a polyclonal antiserum against GBM-HSPG does not cross-react with perlecan. Thirdly, observations from other groups also support the presence of multiple extracellular HSPG species in the glomerulus. In one of these investigations, proteoglycan synthesis was studied in cultures of three cell types from calf glomeruli. Epithelial cells excreted immunologically distinct HSPG components (approximately 300 kDa) as compared to endothelial and mesangial cells, which produced a perlecan-type HSPG (molecular mass 500 kDa) [35]. Another study supported the synthesis of perlecan-like HSPG by cultured rat mesangial cells [36]. Finally, partial amino acid sequences of HSPG isolated from bovine and human kidney also supported the presence of HSPG species that are unrelated to perlecan [37, 38].

After hybridization of kidney mRNA, only the full-length perlecan transcript could be distinguished (Fig. 7). Although minor differences in mRNA length cannot be detected by this method, such small rearrangements could not explain the altered immunoreactivity towards mAbs with different domain specificities (this study and [24, 25]). Heparan-sulfate-carrying splice isoforms of perlecan could not be missed through the choice of the probe, because the corresponding exon is required for the presence of HS chains [20]. Therefore, the production of multiple HSPGs in the GBM is not mediated by alternative splicing of perlecan. This indicates that a different gene is involved.

The availability of the recombinant perlecan-(24–404)-peptide and mAb 95J10 could be of value in determining the functional properties of the individual domains of the perlecan core protein. The ultrastructural localization of perlecan in the GBM suggests that its role in the maintenance of selective glomerular permeability is more modest than was initially assumed. The presence of an additional HSPG, which is unrelated to perlecan, may open a new direction for investigating the structural basis

of charge-selective GBM permeability. The unidentified HSPG that is highly expressed in the GBM may play an important role in renal function.

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